

HBV Pathogenesis

Stefan Wieland, Ph.D.

Hepatitis B virus (HBV) causes acute and chronic necroinflammatory liver disease and hepatocellular carcinoma (HCC). It is widely believed that the outcome of the infection and the pathogenesis of the associated liver disease are determined by host-virus interactions mediated by the immune response. Here we show the results from studies on the host-virus interactions in experimentally HBV-infected chimpanzees. Naïve chimpanzees were inoculated with HBV-positive transgenic mouse serum containing 10^8 genome equivalents (GE) of HBV DNA and virological, immunological, disease, and genomic host-response profiles were monitored throughout the infections.

Pathogenesis of HBV Infection

Monitoring HBcAg-positive hepatocytes by immunohistochemistry and HBV DNA levels by PCR in the liver of the infected chimpanzees showed that virtually 100% of the hepatocytes were infected by HBV at the peak of the infection, and that the infected liver contained approximately 1.1×10^{13} GE of HBV at this time, respectively. However, this massive level of infection was not accompanied by any disease as measured by the release of serum alanine aminotransferase (sALT) into the circulation,⁴ suggesting that HBV gene expression and replication is noncytotoxic to the host, similar to HBV replication in immunologically tolerant HBV-transgenic mice.² Within a couple of weeks after reaching peak infection levels, serum and intrahepatic HBV DNA levels disproportionately decreased compared to the percentage of HBcAg-positive hepatocytes; and there was only a slight increase in sALT levels, suggesting that HBV replication was noncytolytically inhibited. Subsequently, however, sALT activity surged, and HBcAg-positive hepatocytes disappeared during this delayed disease phase. Depletion of CD8⁺ cells during the spread of the infection in one chimpanzee demonstrated that the initial noncytolytic inhibition of HBV replication was dependent on the presence of CD8⁺ cells, suggesting that the IFN γ produced by virus-specific CD8⁺ T cells induces an antiviral mechanism in the hepatocytes similar to what has been observed in HBV-transgenic mice.^{4,6} Furthermore, viral clearance was only achieved when CD8⁺ cell counts rebounded to pretreatment levels, at which point liver disease peaked, as measured by elevated sALT levels, and HBcAg-positive hepatocytes disappeared from the liver.^{4,6}

Genomic Host Response to HBV Infection

RNA samples isolated from liver biopsies harvested over the time course of the infection in three chimpanzees were subjected to DNA microarray analysis⁵ to determine the intrahepatic gene expression profile in the infected livers. Initially, we searched for genes whose expression patterns correlated (directly or inversely) with the amount of HBV DNA in the liver over the entire time course profiled in the HBV-infected chimpanzees. We reasoned that these genes would be regulated by the virus and that they might either reflect activation of the virus-sensing machinery of the cell (i.e., innate immune response), or they might be required for the virus to establish and/or maintain itself in the liver, or both. Surprisingly, no intrahepatic gene correlated with the HBV DNA levels in the liver in the three HBV-infected chimpanzees studied.⁵ Since virtually 100% of the hepatocytes were infected in all three animals, the failure of the virus to induce cellular gene expression as it spread throughout the liver suggests that HBV behaves as a “stealth” virus in that it does not induce an innate response in the cells it infects. Parenthetically, this finding is in stark contrast to the observation that 27 unique transcripts, many of which are known to be stimulated by IFN α/β , fulfilled these criteria in three hepatitis C virus-infected animals, suggesting that HCV induces an innate immune response.³

To identify genes associated with viral clearance, we searched for genes whose expression was induced or suppressed only during the phase of viral clearance in HBV-infected chimpanzees. We identified 110 genes that were induced during viral clearance in HBV-infected chimpanzees, and these genes correlated with virus-specific T cell infiltration and IFN γ expression.⁵ Not surprisingly, the panel of induced genes is heavily weighted towards genes expressed by T cells, and genes known to be induced by IFN γ . Because of the known ability of IFN γ to inhibit HBV *in vivo*, it is possible that one or more of the genes identified may contribute to the noncytopathic clearance of these viruses, similar to the cytokine-mediated antiviral effect on HBV replication observed in the liver of HBV-transgenic mice.^{1,7}

Conclusions

From these studies, it appears that HBV is a noncytopathic virus that establishes itself very efficiently without alerting the innate immune system to its presence by acting as a stealth virus. Furthermore, disease pathogenesis and viral clearance seem to be mediated by CD8⁺ T cells, which seem to exert dual antiviral functions that overlap temporally during natural acute HBV infection: a primarily noncytolytic mechanism, probably mediated by secretion of IFN γ , that inhibits viral replication and a primarily cytolytic mechanism that clears the remaining infected cells.

References

1. Guidotti, L. G., T. Ishikawa, M. V. Hobbs, B. Matzke, R. Schreiber, and F. V. Chisari. 1996. Intracellular inactivation of the hepatitis B virus by cytotoxic T lymphocytes. *Immunity* 4:25-36.
2. Guidotti, L. G., B. Matzke, H. Schaller, and F. V. Chisari. 1995. High-level hepatitis B virus replication in transgenic mice. *J Virol* 69:6158-69.
3. Su, A. I., J. P. Pezacki, L. Wodicka, A. D. Brideau, L. Supekova, R. Thimme, S. Wieland, J. Bukh, R. H. Purcell, P. G. Schultz, and F. V. Chisari. 2002. Genomic analysis of the host response to hepatitis C virus infection. *Proc Natl Acad Sci U S A* 99:15669-74.
4. Thimme, R., S. Wieland, C. Steiger, J. Ghayeb, K. A. Reimann, R. H. Purcell, and F. V. Chisari. 2003. CD8(+) T cells mediate viral clearance and disease pathogenesis during acute hepatitis B virus infection. *J Virol* 77:68-76.
5. Wieland, S., R. Thimme, R. H. Purcell, and F. V. Chisari. 2004. Genomic analysis of the host response to hepatitis B virus infection. *Proc Natl Acad Sci U S A* 101:6669-74.
6. Wieland, S. F., H. C. Spangenberg, R. Thimme, R. H. Purcell, and F. V. Chisari. 2004. Expansion and contraction of the hepatitis B virus transcriptional template in infected chimpanzees. *Proc Natl Acad Sci U S A* 101:2129-34.
7. Wieland, S. F., R. G. Vega, R. Muller, C. F. Evans, B. Hilbush, L. G. Guidotti, J. G. Sutcliffe, P. G. Schultz, and F. V. Chisari. 2003. Searching for interferon-induced genes that inhibit hepatitis B virus replication in transgenic mouse hepatocytes. *J Virol* 77:1227-36.